TOXICOKINETIC MODELLING AND DERIVATION OF THE TWI

CONTAM Opinion on PCDD/Fs and DL-PCBs in food and feed

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Info Session – 13 November 2018

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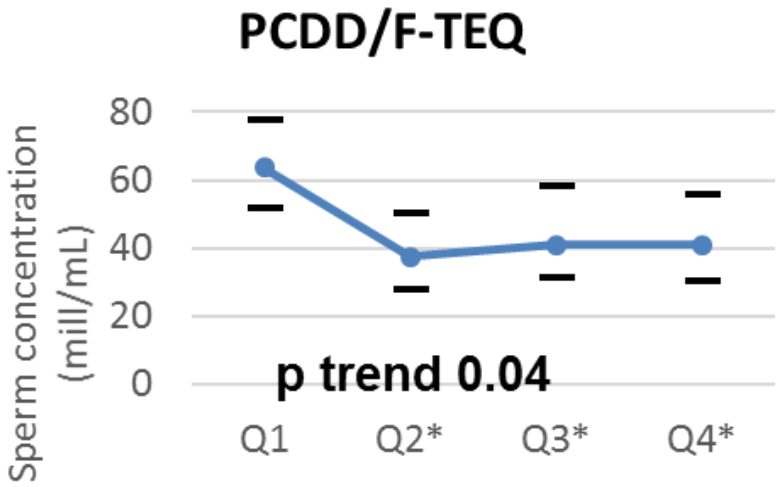
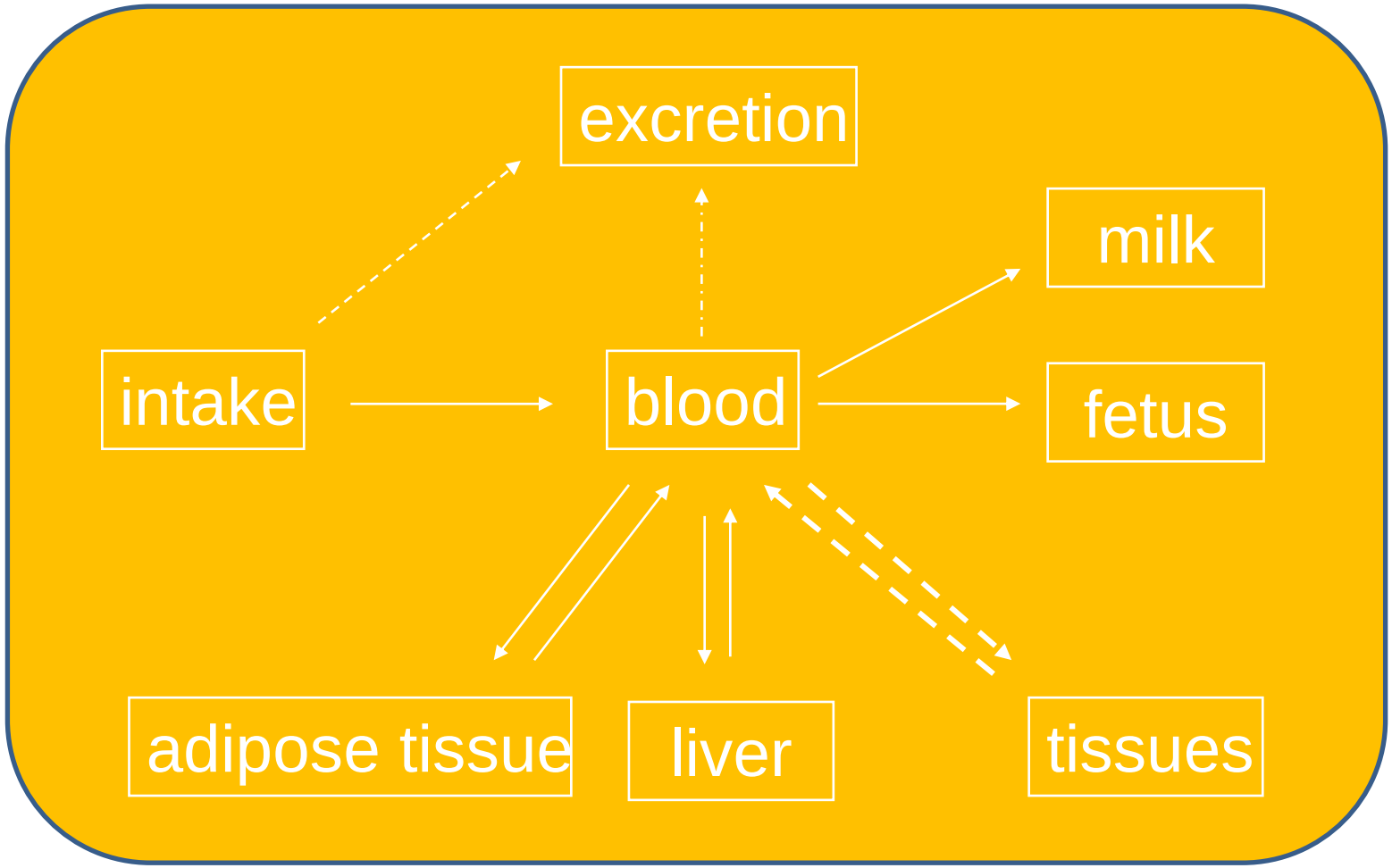
Derivation of the TWI: from NOAEL to EDHI

- NOAEL for serum level PCDD/Fs in Russian boys:
  - at age 9, Q1: 7.0 pg TEQ/g fat
  - Q2 (LOAEL) : 10.9 TEQ/g fat

- Translate this NOAEL for serum to a corresponding Estimated Human Daily Intake (EHDI)



Kinetics in animals and humans



Toxicokinetic modeling

- Toxicokinetic modelling to estimate daily intake corresponding to critical serum level

- SCF used one-compartment model

  - intake (ng/kg bw per day) = (body burden (ng/kg bw) x ln(2)) / ($t_{1/2}$ (days) x f)

  - f: fraction absorbed set at 50%, half-life 7.5 years

Toxicokinetic modeling

- Several toxicokinetic (PBK) models available
  - Two evaluated: CADM and Emond model
- Take into account absorption, metabolism, excretion and distribution to adipose tissue and liver
- Models also take into account CYP1A2 induction, affecting liver sequestration and half-lives
  - Half-life decreases at higher body burdens
  - Liver "storage" increases (liver/fat ratio higher)

Liver sequestration

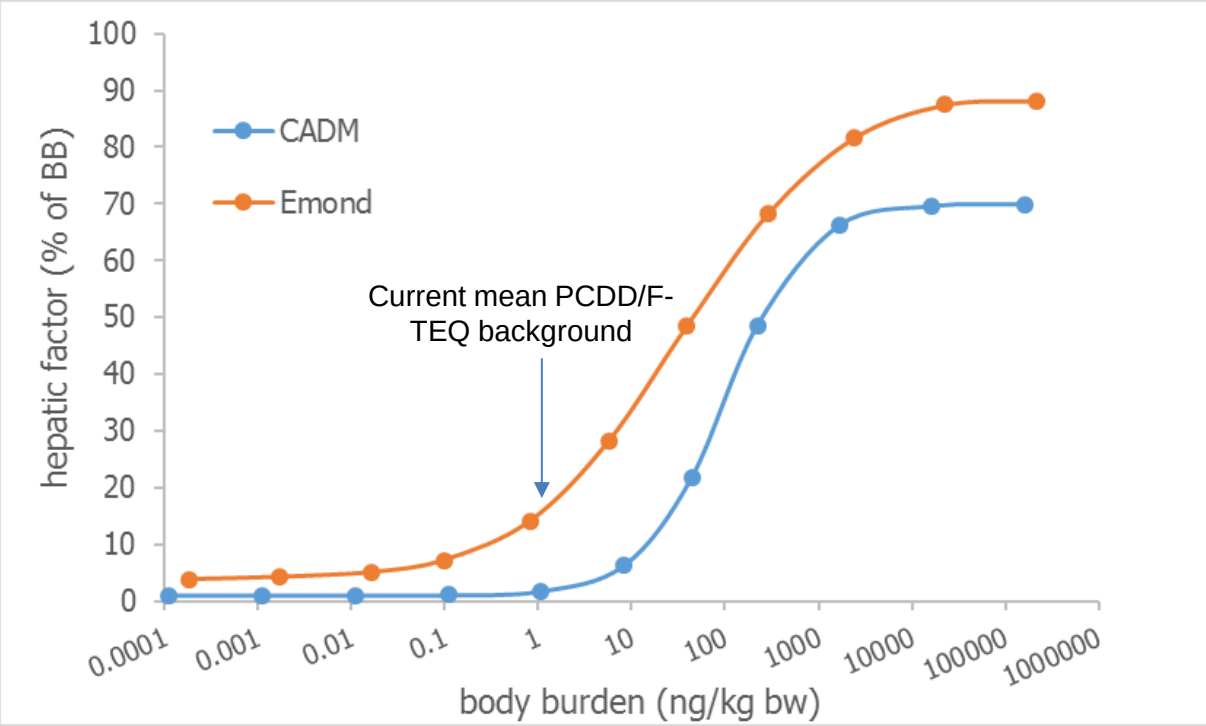


Figure 11. Fraction of the body burden present in the liver, assuming that all TCDD is present in body fat and liver.

Calculations for a 35 year old woman, using the models developed by Emond et al. (2005) and CADM developed by Carrier et al. (1995) and optimised by Aylward et al. (2005). EC50s for the fraction in liver around body burdens of respectively 50 and 100 ng/kg bw, corresponding to body fat levels of 95 and 260 ng/kg fat.

Note: sequestration differs between congeners

Emond model

- Most complete model:
  - Model uses serum levels as dose metric
  - Calibrated on several human cases (*depletion phase*)
- Model translated into Berkeley-Madonna and R
- Results compared to human data:
  - Ratio lipid-based levels serum/adipose tissue too high (2.7)
  - Too high liver levels at low exposure
  - Further adjustments and recalibration required
- Therefore decided not to apply this model

CADM toxicokinetic model

- Concentration and Age Dependent Model (CADM)
  - Developed by Carrier (1995), modified by Aylward (2005), codes for Berkeley-Madonna from Ruiz et al. (2014)
  - Relatively simple model focussing on levels in adipose tissue and liver only (lipid adjusted serum levels assumed equal to adipose tissue based on studies)
  - Taking into account CYP1A2 induction,
  - But also lipid loss into GI-tract (affects half-life at low dose)
- Some adjustments required

CADM toxicokinetic model

Various modifications introduced in codes Ruiz et al. (2014):

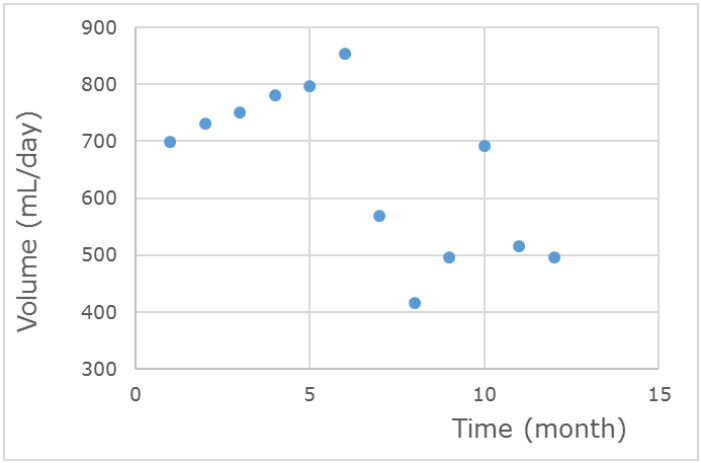
- Growth curve improved
- Exposure based on body weight, so not constant per day
- Higher absorption small children
- Half-life in children assumed to be similar to adults
- Starting level at birth introduced
- During lactation 800 mL/day (not variable over time) (volume based on WHO during first 6 months)

Breast feeding daily volumes (Butte et al., 2002)

Table 2.2: Breastmilk consumption of exclusively breastfed infants 0-6 months of age from developed countries (g/day). Source: Butte *et al.*, 2002.
http://www.who.int/nutrition/publications/infantfeeding/9241562110/en/).

Age, months	1	2	3	4	5	6
Mean, weighted for sample size	699	731	751	780	796	854
Pooled SD[1]	134	132	130	138	141	118
N	186	354	376	257	131	93
Number of study groups	11	14	17	13	10	8

[1]SD=standard deviation.



First 6 months 800 mL OK with data.

After that an overestimation.

Average over 12 months is 650 mL/day.

Table 2.4: Breastmilk consumption of partially breastfed infants 7-12 months of age from developed countries (g/day). Source: Butte *et al.*, 2002.
http://www.who.int/nutrition/publications/infantfeeding/9241562110/en/).

Age, months	7	8	9	10	11	12
Mean, weighted for sample size	569	417	497	691	516	497
Pooled SD[1]	188	226	249	233	215	238
N	251	123	154	5	6	48
Number of study groups	11	8	11	1	1	4

[1]SD=standard deviation.

Breast feeding duration

- WHO: "Exclusive breastfeeding is recommended up to 6 months of age, with continued breastfeeding along with appropriate complementary foods up to two years of age or beyond." (https://www.who.int/topics/breastfeeding/en/)
- Opinion of the Steering Committee of the Norwegian Scientific Committee for Food Safety. Benefit and risk assessment of breastmilk for infant health in NorwayVKM Report 2013: 44
  https://www.vkm.no/download/18.2994e95b15cc5450716157e6/1501690194476/820a1a0bf8.pdf

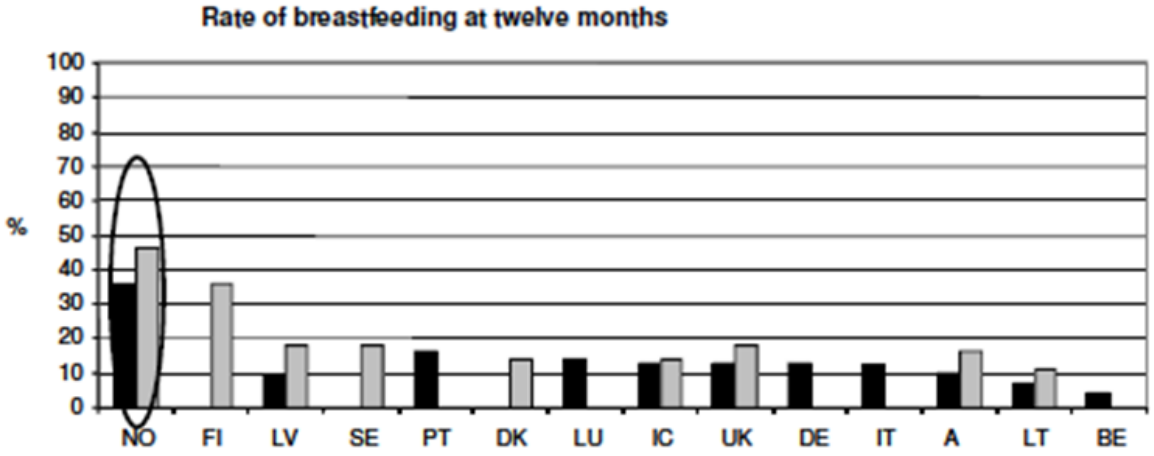


Figure 2.5: Rates of any breastfeeding at 12 months in Europe: 1998-2002 ■ and 2003-2007 ■. Based on Cattaneo *et al*., 2010 and data from Spedkost 2006-2007.

NO: Norway, FI: Finland, LV: Latvia, SE: Sweden, PT: Portugal, DK: Denmark, LU: Luxembourg, IC: Iceland, UK: United Kingdom, DE: Germany, IT: Italy, AT: Austria, LT: Lithuania, BE: Belgium.

Toxicokinetic modeling

- Considerations:
  - NOAEL for serum PCDD/F-TEQ level in 9-years-old boys
  - Serum level affected by higher intake by children via food, but also *in utero* exposure and breastfeeding
  - So levels in mother relevant for those in children
  - Requires calibration between mothers and boys

- Therefore TWI set for adults (mothers), taking into account:
  - two-fold higher intake via food by children
  - *in utero* exposure and breastfeeding during 12 months

- TWI not applicable for evaluating exposure of children during breastfeeding
  - For young children 2x TWI should be used

Outcome modelling boys

Figure 14. Serum level (Ca) in boys, breastfed for 12 months with milk with **5.9** pg/g fat (800 mL per day, 3.5% fat), followed by an intake of **0.5** pg/kg bw per day for an additional 8 years, resulting in a serum level of 7 pg/g fat.

Outcome modelling mothers

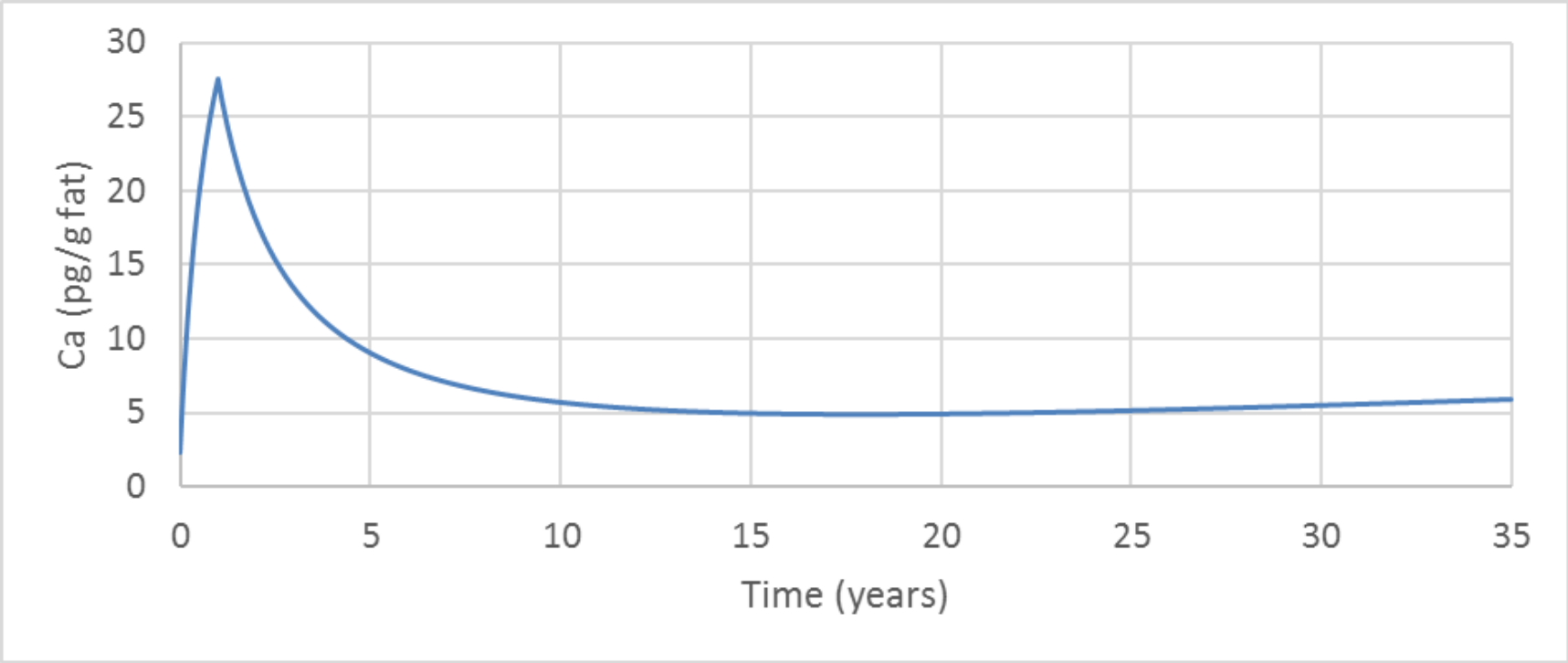


Figure 13. Serum levels (Ca) in a woman, breastfed for 12 months in infancy with milk containing **5.9** pg/g fat, and then being exposed to **0.25** pg/kg bw per day for 34 years.

So milk and fat level at birth of son and start of breastfeeding at 35 years: 5.9 pg/g fat: **input for modelling boys**

Uncertainties applied CADM model

- Model for TCDD but assumed to apply for TEQ
  - Main contributors to exposure PCB-126 (54.7%), 23478-PeCDF (10.7%), PeCDD (7.4%), TCDF (4.9%), TCDD (3.4%), PCB-169 (3.7%) and PCB-118 (2.7%)

- Different half-lives for these congeners: affects accumulation and serum levels
  - TCDD: 6.1-11.3 years across studies (longer at low BB)
  - For PeCDD vs TCDD 10.7 vs 6.5 and 15.7 vs 7.2 years (Aylward et al., 2013, Flesh-Janys et al. 1996)
  - For 2,3,4,7,8-PeCDF vs TCDD 19.6 vs 7.2 and 13.9 vs 9.2 years (Flesh-Janys et al. 1996, Rohde et al. (1999)
  - For PCB-126 vs TCDD 2.7 vs 6.7 years (Ogura 2004)
  - Also shorter half-lives for TCDF and 1,2,3,7,8-PeCDF

Uncertainties applied CADM model

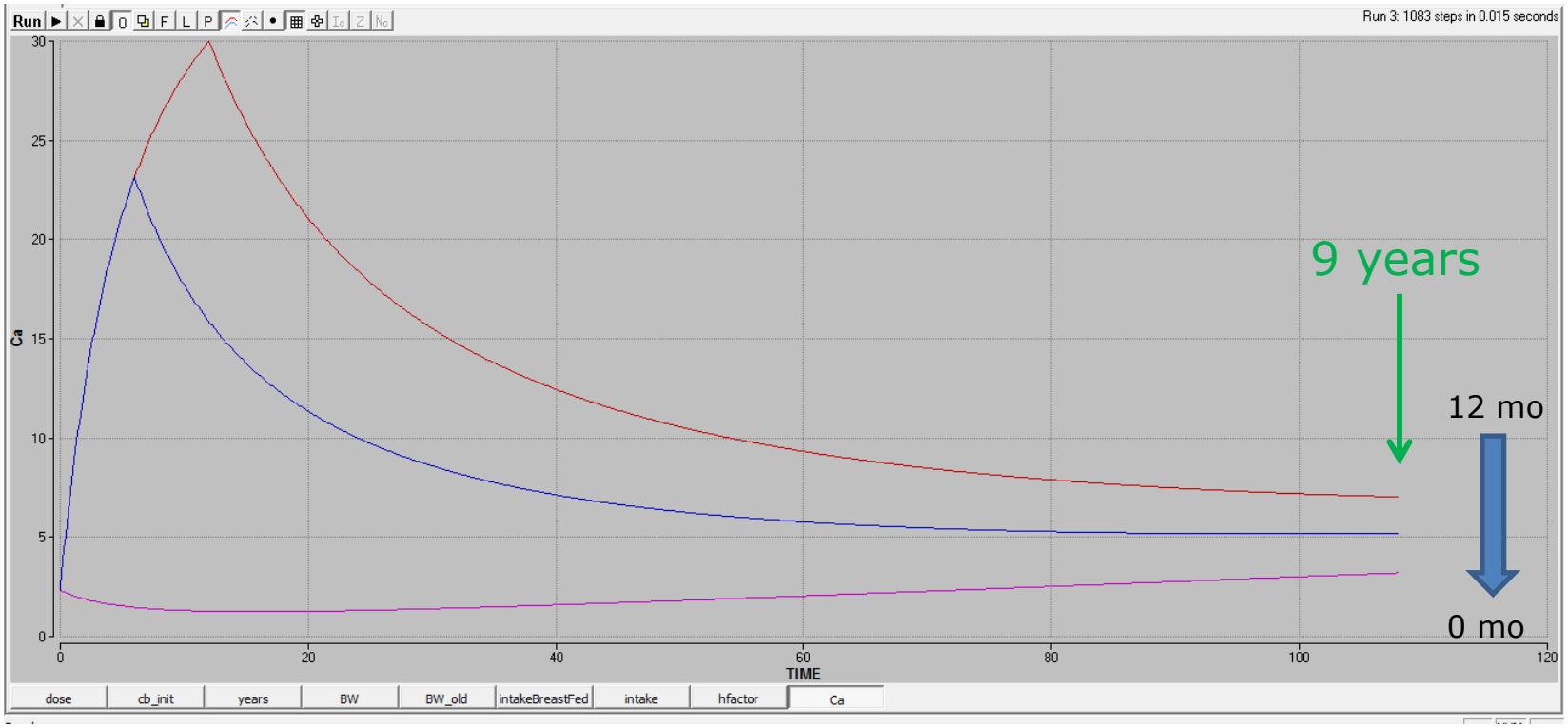
- Possible decrease in milk level during lactation not taken into account

- Constant daily milk volume over the whole period of 12 months

Choice for 12 months breastfeeding and 800 mL per day

- What about shorter/longer duration of breastfeeding?
- What about decrease milk levels due to breast feeding?
- What about decreasing daily intake of milk?

Effect duration of breastfeeding on serum levels boys

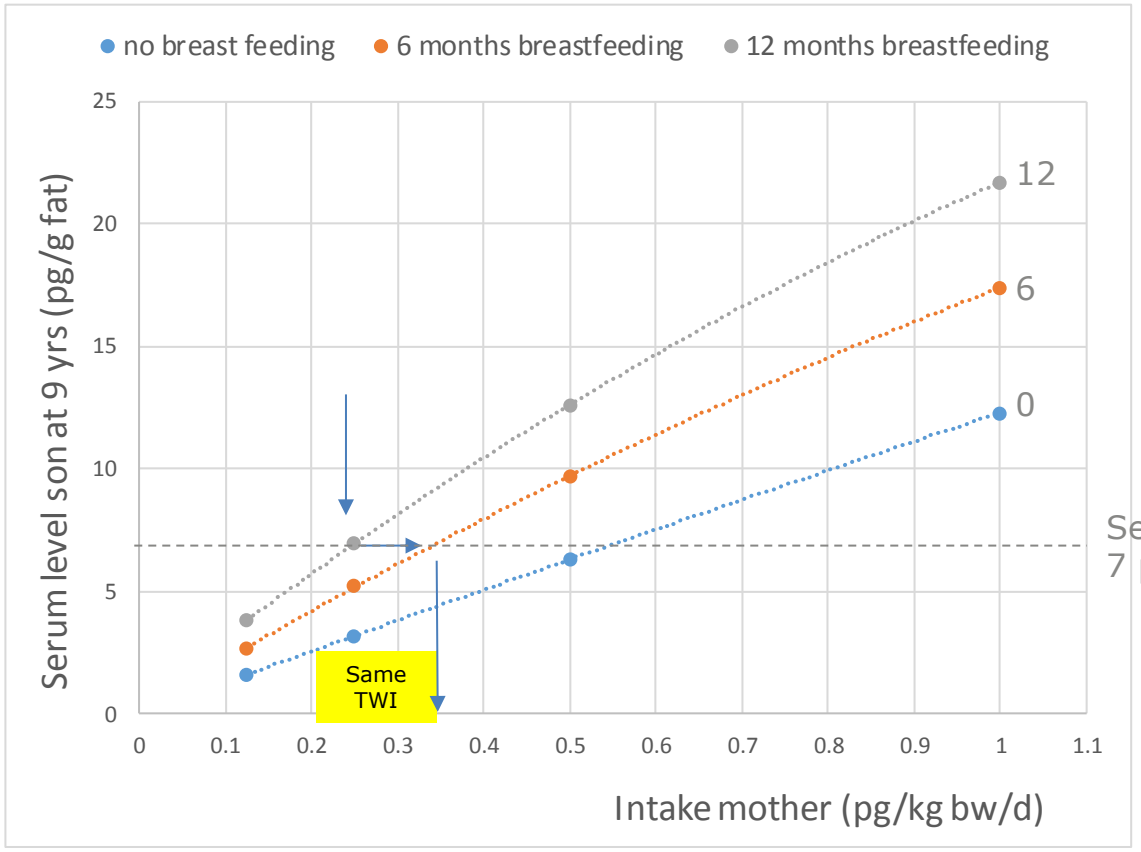


- Effect of 0, 6 and 12 months of breastfeeding on serum levels in boys up to 9 years (milk level 5.9 pg/g fat)

Varying input parameters (Table 15): ⟹ graph

Intake of mothers (pg/kg bw per day)	Human milk level (pg/g fat) [a]	Breast feeding duration (months)	Exposure of boys after weaning similar to mothers		Exposure boys after weaning double of mothers	
			Intake by boys (pg/kg bw per day)	Serum level in boys at 9 years (pg/g fat)	Intake by boys (pg/kg bw per day)	Serum level in boys at 9 years (pg/g fat)
0.13	3.1	0 6 12	0.13	0.8 2.0 3.0	0.25	1.6 2.7 3.8
0.25	**5.9**	0 6 **12**	**0.25**	1.7 3.7 5.6	**0.5**	3.2 5.2 7.0
0.5	10.9	0 6 12	0.5	3.3 6.8 9.9	1	6.3 9.7 12.6
1	19.2	0 6 12	1	6.5 12.0 16.6	2	12.3 17.4 21.7
2	32.5	0 6 12	2	12.5 20.4 26.5	4	23.3 30.2 35.7

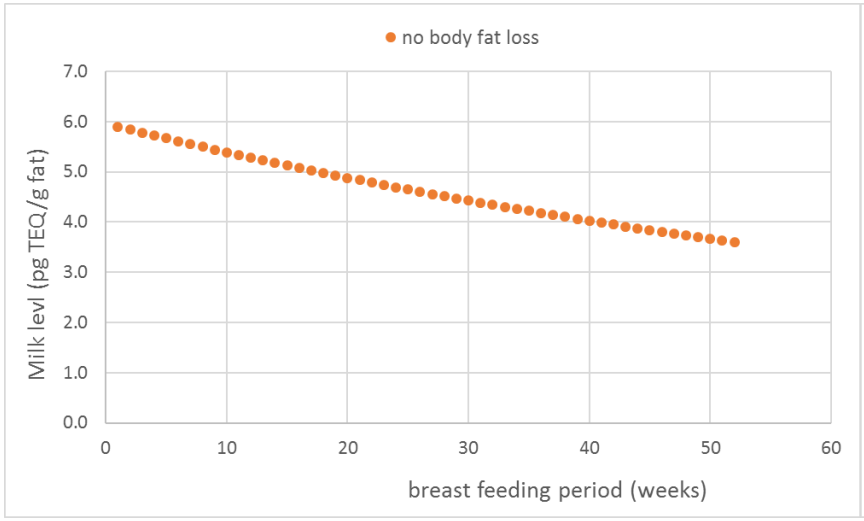
Effect shorter breast feeding duration

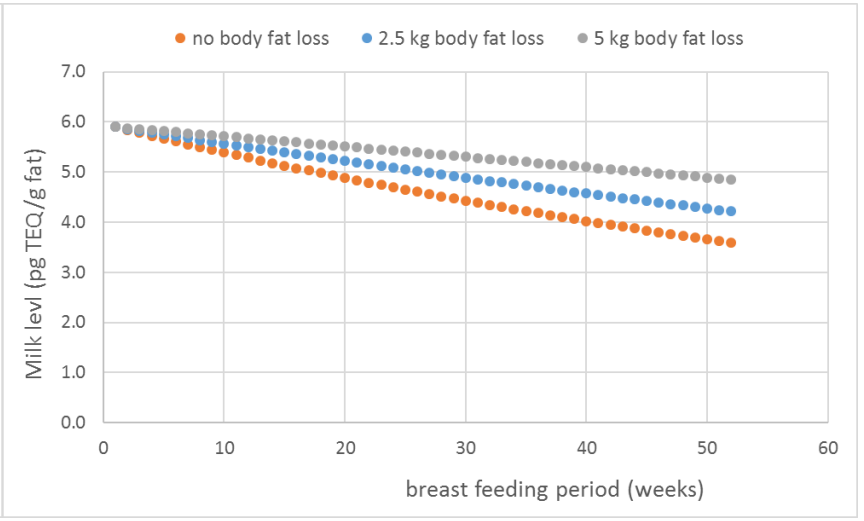


- Increase in boy's serum level between 6 and 12 months 35% (at 0.25 pg/kg bw/day)
- For 6 months of breastfeeding NOAEL of 7 pg TEQ/g fat obtained with mother's exposure of 0.35 pg/kg bw/day
- 0.35 pg/kg bw/day means 2.45 pg/kg bw/week (rounded to 2)

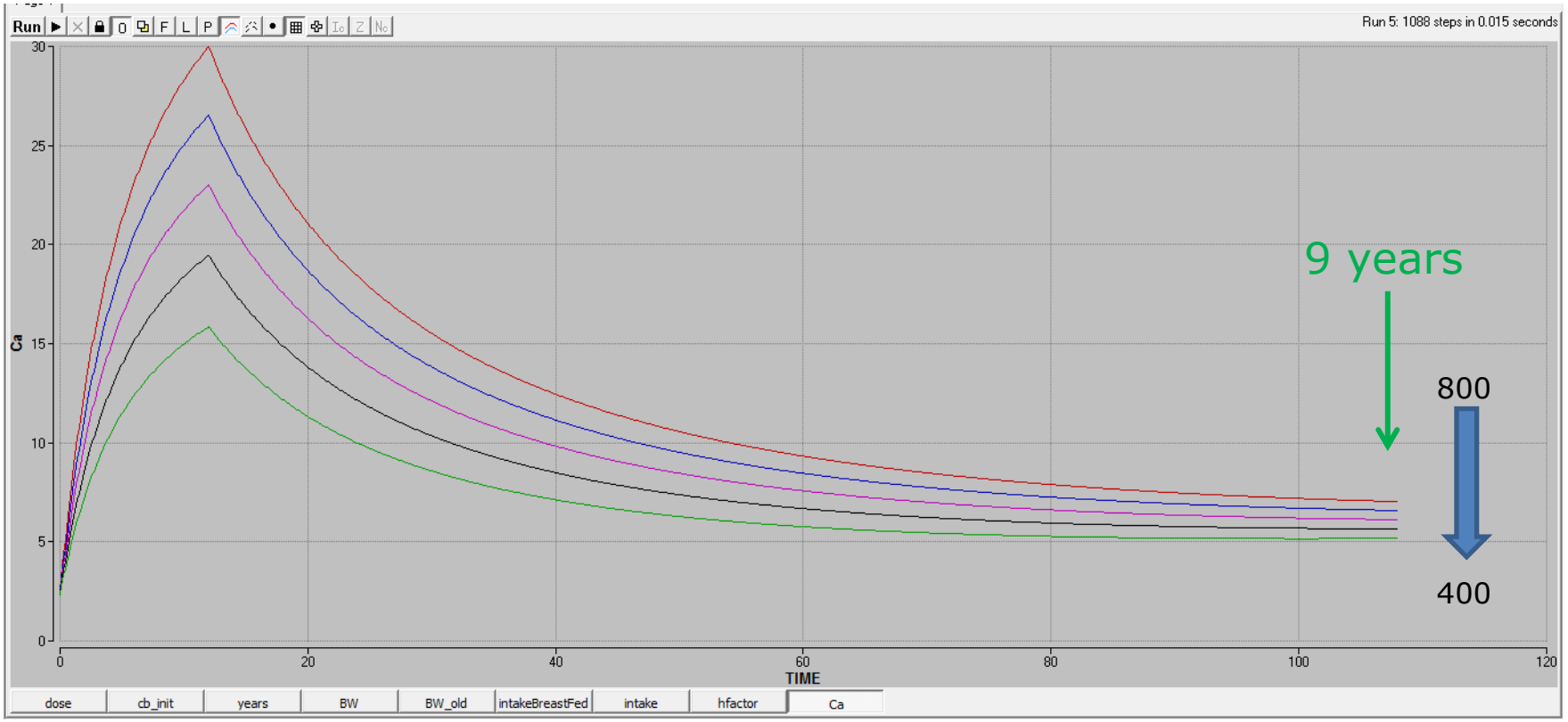
Decrease of milk levels during lactation

- Loss of PCDD/Fs due to lactation,
  - will result in lower absolute body burden of mother
  - thus lower levels in body fat
  - and as a result also lower levels in milk
  - and thus decreasing exposure baby
- Effect smaller when also a loss of body fat
- Decrease observed in some studies
- Not taken into account: recommendation to optimize model(s)



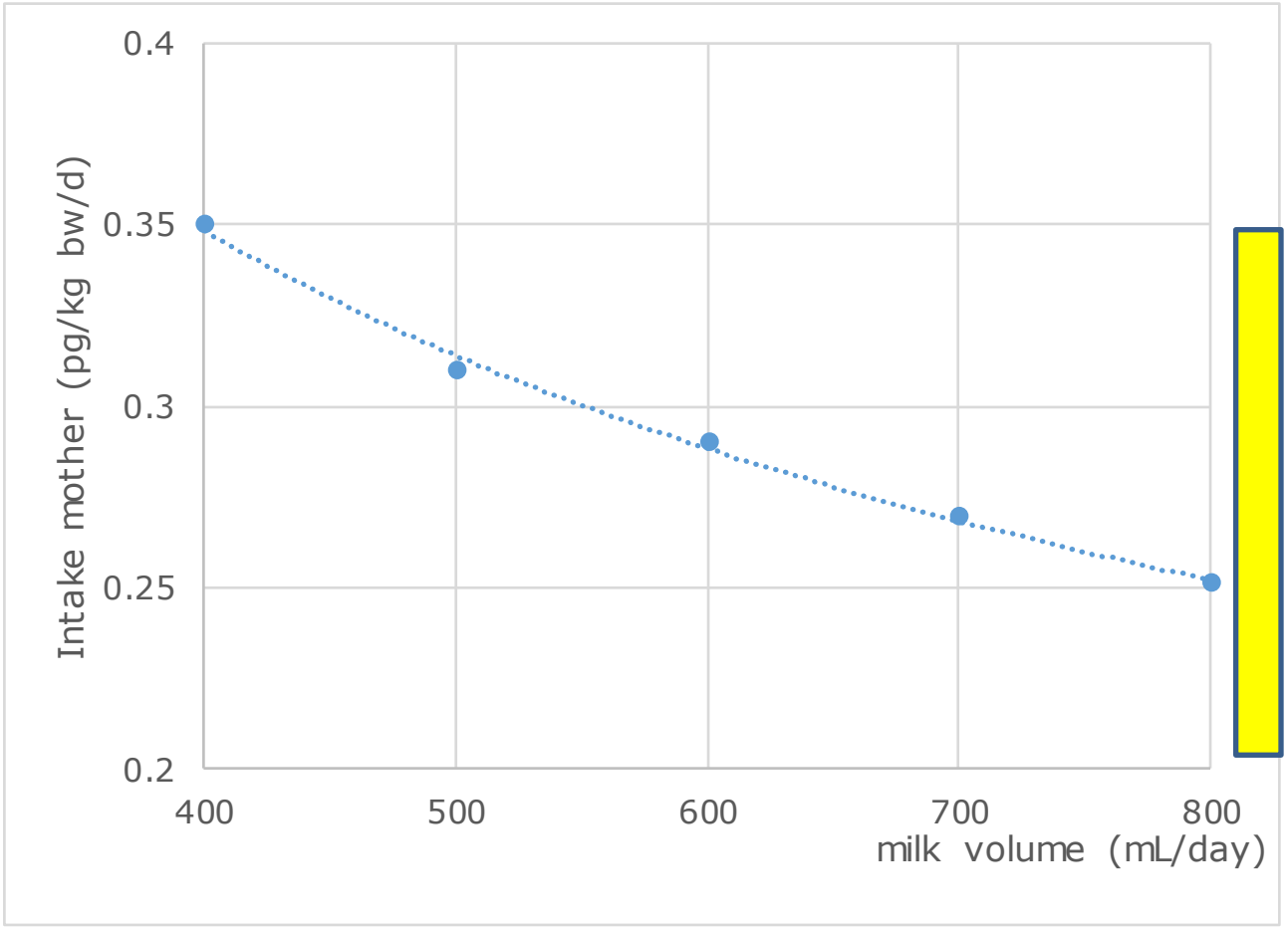


Effect milk intake per day on serum levels boys



Effect milk intake of 400, 500, 600, 700 and 800 mL/day during 12 months, resulting in serum levels at age 9 years of 5.2, 5.7, 6.1, 6.6 and 7.0 pg/g fat

Effect daily human milk consumption on TWI



- Intake mothers leading to serum level boys of 7 pg/g fat
- Average 600 mL/day in 12 mo
- Range leading to TWI of 2 pg/kg/week is 0.21 to 0.35 pg/kg bw/day
- Therefore impact overestimation milk volume per day on TWI seems small

Setting of the TWI

- Decision to maintain weekly base
  - Peak exposure could lead to high exposure baby
  - Not covered by toxicokinetic models
  - Unclear if high intake spread over longer period (month, year) is safe
  - Scenarios described in Appendix F

- TWI rounded to **2 pg TEQ/kg bw/week**
  - So sevenfold lower than previous TWI set by SCF (2001)
  - Lower TWI partly due to different toxicokinetics: higher absorption, longer half-life at lower body burden
  - Similar results when using the previously applied one-compartment model (*not included in Opinion*)

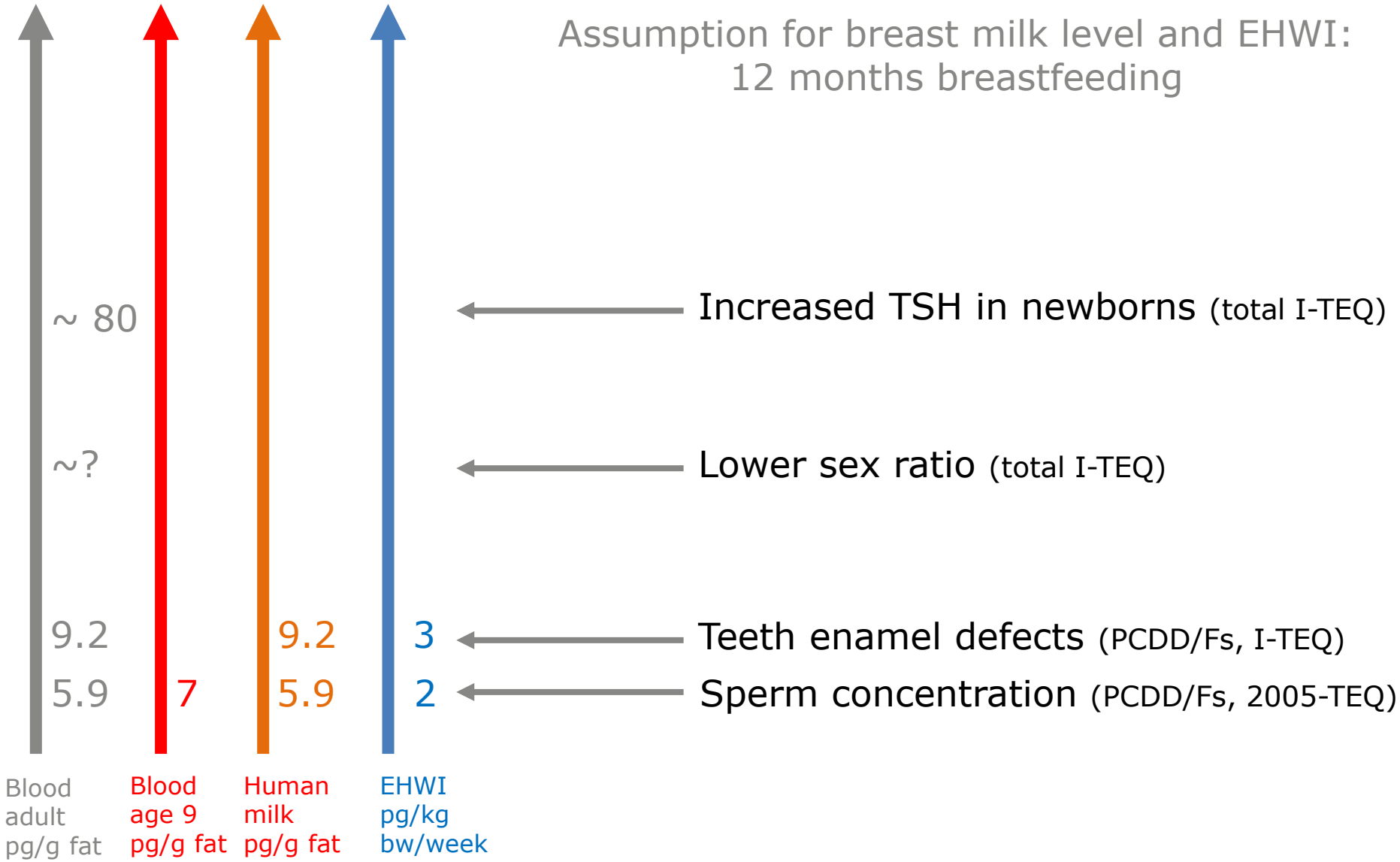
Conclusions modelling human studies

- Milk level of 5.9 pg/g fat at an intake of 800 mL/day for 12 months results in serum level of 7 pg/g fat in 9-years-old boys
  - Corresponding to intake of 0.25 pg/kg bw/day by mothers
  - And two-fold higher intake by boys, i.e. 0.5 pg/kg bw/day

- This intake is rounded to a **TWI of 2 pg TEQ/kg bw/week**

- TWI is fairly robust for shorter duration of breastfeeding and lower milk consumption per day

Different endpoints – serum NOAELs and EHDI



What about animal studies?

- Faqi et al. (1998) (sperm effects male offspring):
  - BB LOAEL of 25 ng/kg bw
  - UF of 3 gives NOAEL of 8.4 ng/kg bw
  - UF 3.2 intraspecies differences: 2.6 ng/kg bw
- Applying CADM: Estimated Daily Human Intake 0.46 pg/kg bw/day
  - Would be rounded to TWI of 3 pg/kg bw/week
- Bone effects at $BMDL_{10}$ of 14 ng/kg bw (Jämsä et al. 2001)
  - UF of 3.2 gives 4.3 ng/kg bw
  - So slightly higher than 2.6 for sperm effects

Conclusions modelling animal studies

- Critical studies in laboratory animals result in TWIs close to those derived from the Russian Children's Study

- This is due to different assumptions in modelling like higher absorption and longer half-life at low body burdens